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BIO HARDENING IN MICROPROPAGATION

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ABSTRACT

Acclimatization is one of the bottleneck in in vitro grown plantlets. A process in which micropropagated plantlets are inoculated with suitable strains of microorganisms in order to enhance their acclimatization. Scientifically defined as 'Biotization/Bio-hardening is a metabolic response of in vitro grown plant material to a microbial inoculant(s), leading to the developmental and physiological changes enhancing biotic and abiotic stress resistance of the derived propagules. VAM is widely using bioagent because of its intracellular obligate endosymbiont nature, improves the properties of soil in rhizophere, enlarges root areas of host plants and improves its efficiency of water absorption and enhances the absorption of P and other nutritional elements. AMF (Arbuscular Mycorrhizal fungi) i.e VAM and Plant Growth Promoting Bacteria are gaining much importance as this concern. Glomus mosseae, Glomus manihotis, Gigaspora ramisporophora, Scuttellospora fulgida and Enterophoraspora colombiana are important AMFs and Azospirrulum spp. Azotobacter spp. Bacillus spp. Bradyrhizobium spp. Pseudomonas sp. are the important PGPRs used in hardening of micropropagated plants. Bio agents can be applied during in vitro stage (inoculation through media) or during acclimatization stage (soil, drip irrigation, spray or root dipping). The bio-agents such as VAM, bacteria and others are reported to substantially enhance the establishment rate of the micropropagated plants and they empower the plants with extra molecular weapons to tackle the situation once exposed to the ambient environment. Hence to get beneficial effects of these organisms and it is better to exploit much of these species in micropropagation of commercial horticultural crops.

KEYWORDS: Biohardenig, AMF, VAM and PGPRs

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INTRODUCTION

Micropropagation is a one in which a tissue is taken from a plant and grown in *in-vitro* condition to produce a large number of plantlets which are genetically identical to the parent. Which is carried out completely under aseptic condition. It mainly involves; initiation (establishment) of the culture, multiplication and root regeneration of initiated/established cultures and acclimatization of regenerated plantlets. Efforts have been directed to optimize the conditions for *in vitro* stages of micropropagation, but acclimatization is major drawback of micropropagated plants. Consequently, the transplantation stage continues to be a major bottleneck in the micropropagation of many plants. Plantlets or shoots that have grown *in vitro* have been continuously exposed to a unique microenvironment that has been selected to provide minimal stress and optimum conditions for plant multiplication. The acclimatization of plantlets is mainly depends on its physiological and anatomical characteristics. It is very important to maintain the cultures after the rooting before transplanting them dierectly to

open environment should be maintained in green house so that gradual acclimatization of plantlets can be achieved. In present study highlighted about role of bioagents in acclimatization rather normal media.

MATERIAL AND METHODS

Regenerated Explants

Study carried out in regenerated healthy explants of pomegranate (*Punica granatum* L.) cv. 'Bhagwa', collected from two year old healthy and vigorously growing mother plant.

Initiation

Pre-treated and surface sterilized explants were inoculated in initiation medium (full strength MS media containing 3% sucrose, 35 mg/l adenine sulfate, 35 mg/l citric acid and 1.5 mg/l BAP) for aseptic culture establishment on

Multiplication and Sub Culturing

The inoculated explants and cultures were transferred on to a fresh multiplication medium (MS B + BAP 1.00 mg/l + $AgNO_3$ 0.50mg/l and MS B + BAP 1.00 mg/l + $AgNO_3$ 1.00mg/l). sub culturing was carried out every 25-30 days after incubation to minimize problems like browning, vitrification and contamination.

Rooting of Regenerated Shoots

The regenerated healthy explants were transferred on to a fresh rooting medium (half strength MS B +IBA 1.00 mg/l).

Acclimitization

The regenerated rooted plants were transplanted to soil (As a control) and different spp. of AMFs and PGPRs.

RESULTS

Results are furnished in **Table 1.** on basis of scoring by visual observations.

The regenerated healthy explants were responded superior in *Glomus mosseae* and *Azospirrulum spp* followed by *Scuttellospora fulgida* and *Pseudomonas spp.*, in which response was quite better as compare to control. Other species also responded good to average as compared to control. Present study substantiated by Singh *et al.*,2012 in pomegranate, Singh *et al.*,2011 in grape, Lorenzo *et al*. 2010 in **rootstock** Mr.S 2/5 and Vasane and Kothari, 2006 in banana.

DISCUSSIONS

In the present study, the bio agents played a vital role in acclimatization of micropropagated plantlets of pomegranate. AMF and PGPR strains were significantly superior over the control with regard to growth, physiological and biochemical attributes and survivility was also superior with respect to treated plantlets.

CONCLUSIONS

Thus present study concludes that; bioagents substantially enhance the establishment rate of the micropropagated plants and they empower the plants with extra molecular weapons to tackle the situation once exposed to the ambient environment. Hence beneficial effects of these organisms must be exploited more in commercial microporpagation of many horticultural crops.

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APPENDICES

Table 1: Survival of Explants in Vivo Condition in Presence of Different Spp. of Bio Agents

Treatments	Bio Agents	Species	Results /Response
T_1	**Control**	Soil	-
T_2	**Arbuscular Mycorrhizal fungi (AMF)**	*Glomus mosseae*	++++
		Gigaspora ramisporophora	++
		Scuttellospora fulgida	+++
		Enterophoraspora colombiana	++
T_3	**Plant Growth Promoting Rhizobacteria PR strains**	*Azospirrulum spp.*	++++
		Azotobacter spp.	++
		Bacillus spp.	+
		Bradyrhizobium spp.	++
		Pseudomonas spp.	+++

++++: Superior, +++: Better, ++: Good, +: Average, -:Nil